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Addressing spatial variability in provenance experiments exemplified in two trials with black spruce

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ABSTRACT: Two exemplary black spruce (*Picea mariana* [Mill.] B.S.P.) provenance trials were analyzed using traditional and spatial techniques. The objective was to find out possible differences between these approaches in terms of both the resulting fit-statistics and the estimated mean heights of provenances. Further, the spatial model was consequently adjusted to treat global and extraneous sources of variation. As expected, models incorporating spatial variation provided a better fit to the data. Consequently, there was also a noticeable shift in ranking of individual provenances, which has an important implication for the interpretation of provenance experiments results. Problems associated with the analysis of traditional randomized block designs in forestry research are discussed.

Keywords: *Picea mariana* (Mill.); provenance research; REML; spatial variation

Most variety trials utilize complete or incomplete block designs and are analyzed with the traditional analysis of variance (ANOVA). The last decade of the 20th century saw major improvements in the options available for the analysis of field trials. Experience with many analyses lead to the realization that spatial variation has multiple sources and classical field designs often fail to do justice to the spatial variability (Cadena et al. 2000). Though forest genetic trials are similar to agricultural variety field trials, there are a number of differences. Forestry trials are often much larger because of the large size of individual plants and the higher replication needed to achieve satisfactory family estimates (Dutkowski et al. 2002). The size of forestry trials is also magnified by including large numbers of genetic entries (e.g. clones, provenances), often leading to inefficient blocking due to large site heterogeneity within blocks. Further, individual trees are usually regarded as uncorrelated to the neighboring ones, although competition among them is supposed to be more important than inter-plot competition in variety trials. The most common type of experimental design in provenance research is the randomized block design. More recently, spatial analytical methods have been used to study patterns of site variation (Fu et al. 1999) and have been shown to improve the precision of estimated effects for provenances (Hamann et al. 2002), or clones (Costa E Silva et al. 2001) in forest tree breeding trials.

Recently, a number of statistical approaches became available to ordinary users due to emerging increase in the power of personal computers. As a result, mixed models can be implemented with properly declared factors having either fixed or random effects. Further, it is possible to investigate various error correlation structures in the supplied data. Local or global trends in site variability can be efficiently strained away through their proper declaration in the statistical model. The main objective of classical provenance trials is to obtain precise estimates of provenance means and/or their respective contrasts. Soil fertility, soil water-holding capacity, soil physical characteristics and other environmental

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factors often vary across an experimental site. Previous history, irrigation, plot trimming, direction of cultivation or harvesting are other man induced sources of variation. The site variability in field trials can be spatially continuous, reflecting similar patterns in underlying soil and microclimatic effects; discontinuous, reflecting cultural or measurement effects; or random, because of micro-environmental heterogeneity. Spatially continuous variation may appear as a local trend (patches) or as a global trend (gradients) over the whole site (Dutkowski et al. 2002). Good experimental design can reduce the impact of some of these factors but unless they are appropriately included in the statistical model when they occur, they will result in poor precision in estimates of variety effects and variety contrasts (Cadena et al. 2000).

Nevertheless, it is a challenging task to analyze inappropriately established provenance experiments. Littell et al. (1996) argue that "Spatial analysis is not a cure-all. Good experimental design is essential". Unfortunately, many tree breeding trials do not utilize more efficient experimental design layouts and rely on rather simple schemes. Often, the type II error rate is not considered while the experiment is established leading to either:

(1) insufficient power of the test, or
(2) very large experiments with inappropriate control of the site's heterogeneity.

The objective of this study is to outline some methodical problems associated with the statistical evaluation of provenance experiments. Though the problems might be considered general, an example is used in this paper focusing on a provenance test with black spruce in the Czech Republic. In the background of this experiment, there is a demand for alternative forest tree species from between the 1970's and 1990's. The choice of tree species for reforestation of immission clearings and restoration of forest stands, especially in the Krušné hory Mts. (Ore Mountains) and the Jizera Mountains, represented one of the most difficult tasks in forestry at that time (Vacek et al. 2003; Peřina et al. 1984). Exotic spruce and pine species were planted in the most extreme conditions as a trial solution because prosperity of native pioneer tree species could not be granted, often due to damages caused by game (Vacek et al. 1995). Based on the primary results from an international test with several exotic spruce species (evaluation in September 1988), black spruce was chosen for further investigation. It performed best from the viewpoint of both growth parameters and survival, and thus a large provenance test with this species was established in Central Europe in the mid 1990's (Kobliha 1998). In this paper, the main focus was directed at contrasting different conventional and spatial statistical approaches rather than providing a detailed evaluation of the whole provenance experiment.

MATERIALS AND METHODS

Data sets

In 1995, the Saxon Forest Research Institute in Graupa, Germany, initiated a large international provenance test in co-operation with the Forestry and Game Management Research Institute in Jíloviště-Strnady, the Czech Republic. 16 provenance trials were established in the framework of this experiment: 4 trials in Germany, 10 trials in the Czech Republic, and 2 trials in Slovakia. This test consisted in total of 42 Canadian black spruce provenances (provenances 1 to 42), 5 Norway spruce provenances from Germany (provenances 43 to 47), and local Norway spruce provenances (48 and above) acting as the comparative standards. Evaluation of most of these trials was carried out in late April 2005 at the age of 13. Height, breast-height diameter, fructification, frost damages, and damages caused by wildlife were measured on every individual tree. The two provenance trials included in this study were established in spring 1995 using three-year-old plantings. Norway spruce provenances from Germany were sown in 1991, thus they were one year older.

All of these trials were established in accordance to the IUFRO methods using randomized complete block design (RCBD) with four replications. They

Table 1. Selected parameters of two provenance trials evaluated in the current study

Trial	Trial name	Area (ha)	Number of provenances[1]	Blocks[2]	Rows	Columns	Shape	Altitude (m a.s.l.)
1	Přimda	0.72	42-5-3	4 (2)	35	75	rectangle	500
2	Tišnov	0.43	23-0-0	4 (3)	60	80	irregular	370

[1]Number of black spruce provenances – provenances of Norway spruce from Germany – local Norway spruce provenances
[2]Number of replicates (replicates included in this study in brackets)

consist of plots 6 × 6 m; spacing between individuals is 1.2 × 1.2 m. Every plot contains 25 individuals of one provenance; provenances are represented by open-pollinated families.

Data were collected in spring 2005. Growth was measured as height with an accuracy of 1 cm through the use of a telescopic height-finding lath. Other recorded traits were not included in this paper. All dead and missing trees were treated as missing values. For the purposes of this study, two blocks were only used out of the original four in the trial #1, while three blocks were used in the trial #2. The remaining ones had to be excluded from the analysis owing to the survival rate of trees being unsatisfactorily low. Besides, 7 provenances were exempt from the analysis in trial 2 as well due to high standard errors.

Statistical model

The individual tree data from each trial were all analyzed using several linear mixed models of the general form

$$\mathbf{Y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\boldsymbol{\gamma} + \boldsymbol{\varepsilon} \qquad (1)$$

where: $\mathbf{Y}$ – vector of observed values,
$\boldsymbol{\beta}$ – vector of fixed effects with its design matrix $\mathbf{X}$,
$\boldsymbol{\gamma}$ – vector of random effects with its design matrix $\mathbf{Z}$,
$\boldsymbol{\varepsilon}$ – a vector of residuals.

The mixed model extends the general linear model (e.g. procedure GLM in SAS®) by allowing a more flexible specification of the covariance matrix of $\boldsymbol{\varepsilon}$. It is an unknown random error vector whose elements are no longer required to be independent and homogeneous. In other words, it allows for both correlation and heterogeneous variances, although one still assumes normality. The name mixed model comes from the fact that the model contains both fixed-effects parameters, $\boldsymbol{\beta}$, and random-effects parameters, $\boldsymbol{\gamma}$. To further develop this notion of variance modelling, assume that $\boldsymbol{\gamma}$ and $\boldsymbol{\varepsilon}$ are Gaussian random variables that are uncorrelated and have expectations 0 and variances $\mathbf{G}$ and $\mathbf{R}$, respectively. The variance of $\mathbf{Y}$ is thus:

$$V(\mathbf{Y}) = \mathbf{ZGZ}' + \mathbf{R} \qquad (2)$$

where: $\mathbf{R}$ – variance-covariance matrix of the residuals,
$\mathbf{G}$ – direct sum of the variance-covariance matrices of each of the random effects (SAS®Institute Inc. 1999).

Where residuals are supposed to be independent, $\mathbf{R}$ matrix is defined as $\sigma^2_e \mathbf{I}$. Spatial analysis allows the matrix $\mathbf{R}$ to have alternative structures based on the decomposition of $\boldsymbol{\varepsilon}$ into two groups of residuals: spatially dependent (ξ) and spatially independent (η). Covariance structure used in this study assumed separable first order autoregressive processes in rows and columns, for which the $\mathbf{R}$ matrix is:

$$\mathbf{R} = \sigma^2_\xi \, [\mathbf{AR1}\,(\rho_{col}) \otimes \mathbf{AR1}\,(\rho_{row})] + \sigma^2_\eta \mathbf{I} \qquad (3)$$

where: σ^2_ξ – spatial residual variance,
σ^2_η – independent residual variance,
$\mathbf{I}$ – identity matrix,
$\mathbf{AR1}(\rho)$ – stands for a first-order autoregressive correlation matrix where ρ is the autocorrelation parameter to be estimated from the data (Dutkowski et al. 2002).

Original "design" model

Several statistical models were evaluated for each trial. The aim was to achieve high value of the log-likelihood of the fitted model, while controlling standard errors of the estimates. As a base scenario, a traditional design model was implemented, in which the original experimental design features of the trials were fitted. This model is referred to as the randomized complete block design (RCBD) with replicates (blocks) having random effects and provenances having fixed effects. This design model, in which the residuals were spatially independent, was evaluated using SAS PROC MIXED (SAS® Institute Inc. 1999).

Spatial model

Second set of models allowed the modelling of spatial patterns in residual variation. The goal was to reveal possible local and global trends using autoregressive model (Model AR1) where spatially independent residuals (η) were omitted, and thus all the residuals were assumed to be spatially dependent (ξ). For this analysis, we employed a software package ASReml® (Gilmour et al. 2002), which uses the REML (Restricted Maximum Likelihood) estimation method to estimate variance components in the context of mixed linear models. It is a useful tool for analyzing field variety trials as it allows for the fitting of spatial variability within field trials in a variety of ways (Cadena et al. 2000). Sample variograms were created in order to identify spatial variance patterns within the two trials. The sample variogram is a plot of the semi-variances of differences of residuals at particular distances. It is essentially the complement of the spatial autocorrelation matrix but it is easier to view and interpret (Gilmour et al. 2002).

Spatial model with additional sources of variation

Sequential experimental approach to spatial analysis described by Cadena et al. (2000) was followed

Table 2. Covariance parameters estimates along with standard errors, and the Pr Z value (one- or two-tailed area of the standard Gaussian density outside of the Z-value)

	Covariance parameters	Estimate	Standard error	Pr Z
Trial 1	block	303.52	449.43	0.2497
	provenance × block	477.94	148.90	0.0007
	residual	4,241.33	143.09	< 0.0001
Trial 2	block	101.72	147.80	0.2457
	provenance × block	1,067.43	251.43	< 0.0001
	residual	4,370.79	164.45	< 0.0001

next. These authors distinguish between global, extraneous, and natural variation and propose special measures to treat the variation appropriately in the mixed-model framework. First, global variation (major trends across the experiment) can be fitted as linear trends, cubic smoothing splines, row and column contrasts and covariates. Second, extraneous variation is a consequence of experimental operations and may be modelled with random row and column effects. Third, natural variation arises from the differences in soil moisture, soil depth, and other natural causes that are beyond the experimenter's control. The natural variation is best characterized using the autoregressive correlation structure (e.g. AR1 used in this study). The actual analysis (as performed in this study) is based on the sequential evaluation of these sources of variation in variogram. Based on this procedural graphical output, models are continuously improved with respect to the observed data. The best model (model "AR1 Adj") was selected based upon the evaluation of the variogram (no variability structure is present other than the two-dimensional AR1), model REML log-likelihood, and additional fit measures described by the same authors. Following the sequential approach, the resulting models considered random row and column effects (trial #1) and a third-order polynomial (trial #2). These models were selected out of the family of models based on fit criterions described by the same authors. Simpler models were preferred over complex ones.

RESULTS

Table 2 provides output from SAS© MIXED procedure for both trials considering the RCBD model. It is obvious that in both cases the original block design is inefficient (statistically not significant effect of blocks at alpha = 0.05). Type III test of fixed effects revealed that height is significantly affected by provenances (*p* value for provenances was lower than 0.0001 in both trails, not shown in the figure).

With regards to the fit-statistics, log-likelihood decreased slightly after processing data with the AR1 model (Table 3). In the southern part of trial 1, the variogram revealed a conspicuous trough in site variation in the column direction (Fig. 1, left), which corresponded to approximately 15 columns. This phenomenon might be explained by local differences in water regime because part of the trial is waterlogged. Subsequent adjustment of this model with random effects of columns led to an additional increase in log-likelihood, which was now relatively strong. In this case (Fig. 1, right), the resulting vari-

Table 3. Fit-statistics

		Log-likelihood	*F*-increment	Highest standard error	Lowest standard error	Overall SED*
Trial 1	RCB	–8,583.44	11.47	24.35	21.90	26.80
	AR1	–8,575.49	19.58	16.60	13.08	20.03
	AR1 Adj	–8,540.84	26.47	15.76	12.30	18.46
Trial 2	RCB	–5,424.65	4.68	12.46	8.30	10.59
	AR1	–5,408.04	4.19	14.49	11.47	16.84
	AR1 Adj	–5,368.29	5.34	13.33	10.47	15.44

*The overall SED (Standard Error of Difference) is the square root of the average variance of diference between the variety means. Choosing a model on the basis of smallest SED is not recommended because the model is not necessarily fitting the variability present in the data (Gilmour et al. 2002)

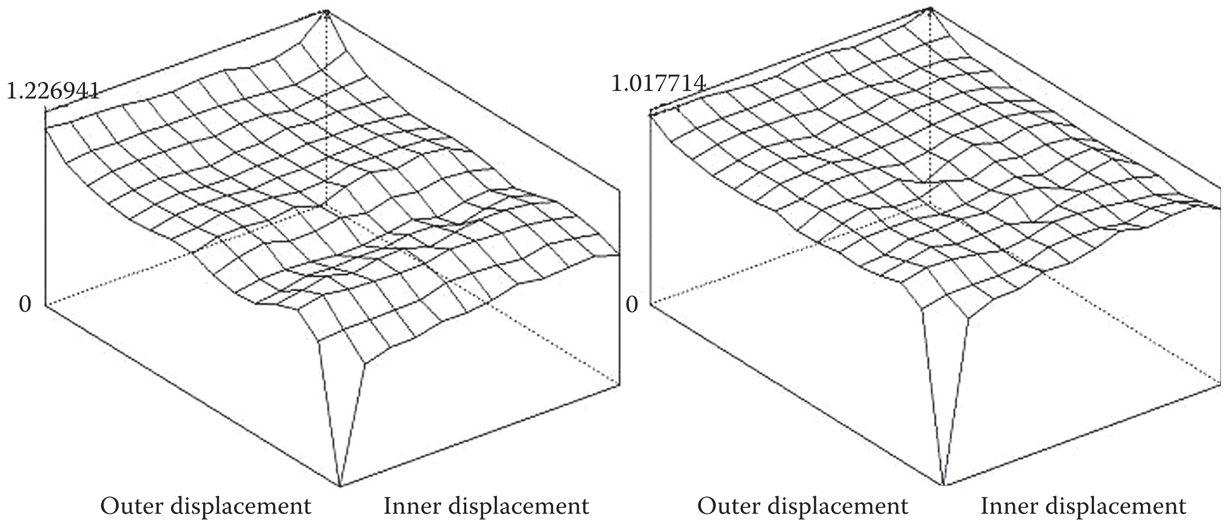

Fig. 1. Variograms of spatial residuals in trial #1 (Přimda) obtained from AR1 (left), and with AR1 Adj (right)

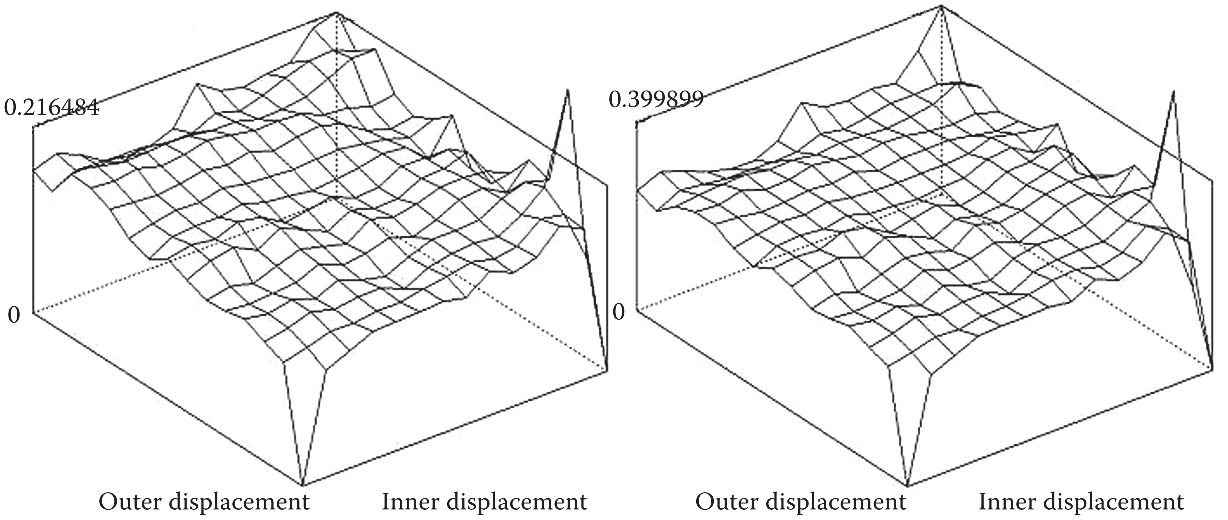

Fig. 2. Variograms of spatial residuals in trial #2 (Tišnov) obtained from AR1 (left), and with subsequent model-fitting with polynomials AR1 Adj (right)

ogram did not show any noticeable gradient as it had been smoothed away. In trial 2, attempts to flatten the primary variogram from AR1 (Fig. 2, left) with random rows and columns failed to produce a variogram indicating stationarity. Though the variogram did not show any gradient, it was quite uneven and contained a lot of local patches. Fitting the AR1 model with polynomials increased significantly the value of log-likelihood as well as *F*-increment (Fig. 2, right).

Not only fit-statistics are affected when different models are used. Predicted mean heights of provenances and their ranking relative to one another for the three models described in the previous chapter are provided in Figs. 3–6. There are a number of apparent differences between these models. In trial 1 (Přimda), provenance #20 performs the best in all cases. However, provenance #15 ranked 15th in the RCBD model (predicted mean height 308.05 cm, standard error 22.18), while it only ranked 29th in the AR1 Adj model (predicted mean height 288.39 cm, standard error 12.81). The opposite effect took place in the case of provenance #28, which was markedly underestimated by the RCBD model. Its order here was 28th (289.83 cm, 24.35), while it reached 309.27 cm (15.76) in the AR1 Adj model. Similarly, in trial 2, there are also significant differences between predicted means as well in relative ranking of provenances. Provenances 11, 20, and 39 seem to be overrated by RCBD; provenances 42 and mainly 13, on the other side, seem to be underrated.

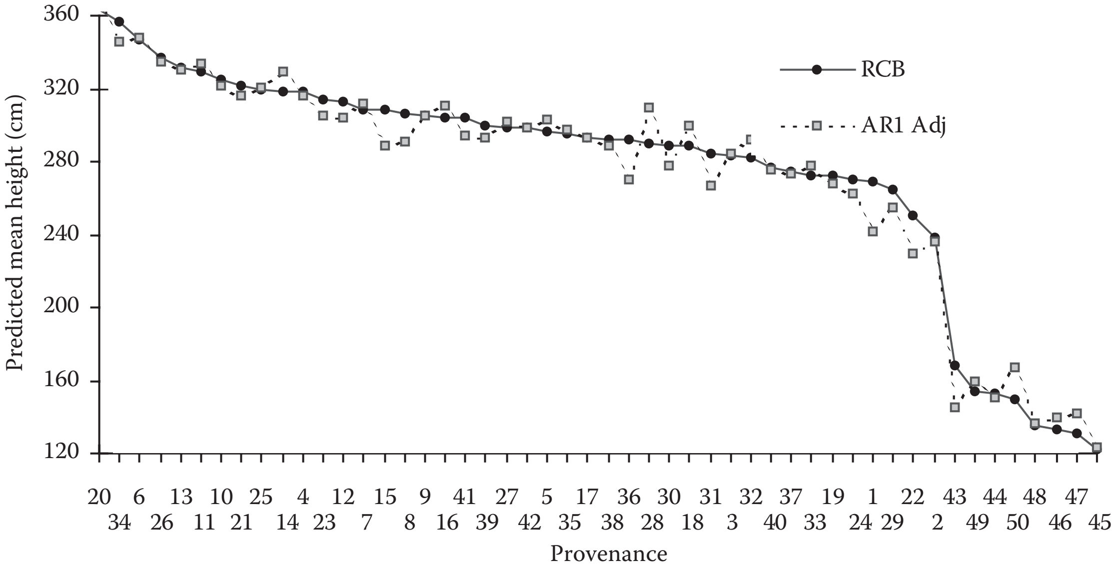

Fig. 3. Predicted mean heights of provenances in trial 1 (Přimda) according to RCBD and AR1 Adj

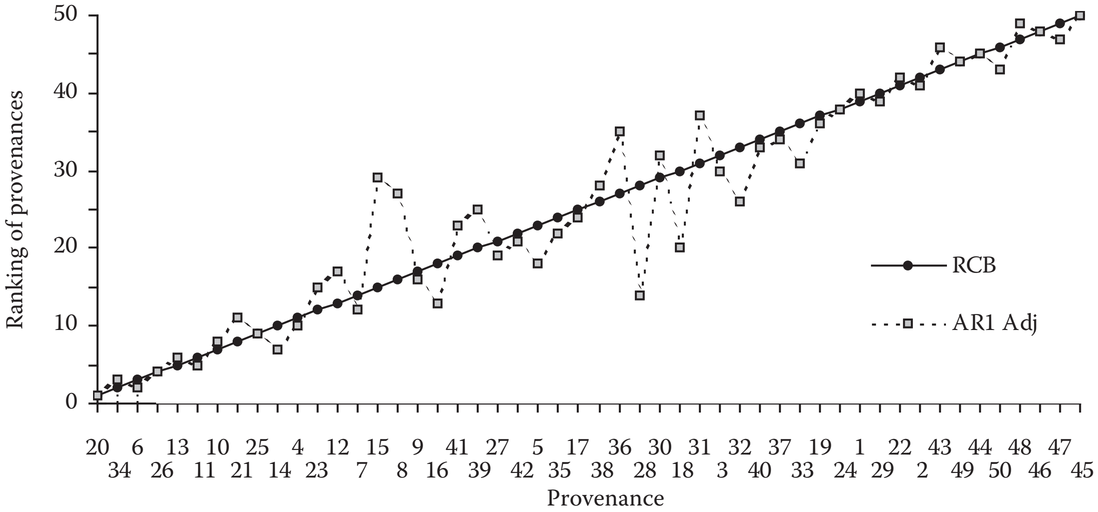

Fig. 4. Ranking of provenances relative to one another in trial 1 (Přimda) according to RCBD and AR1 Adj

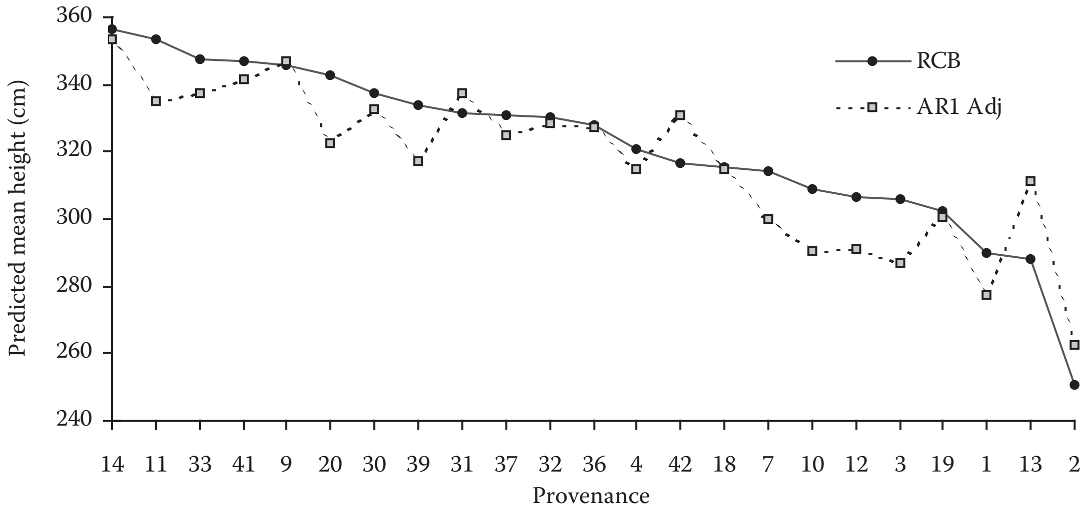

Fig. 5. Predicted mean heights of provenances in trial 2 (Tišnov) according to RCBD and AR1 Adj

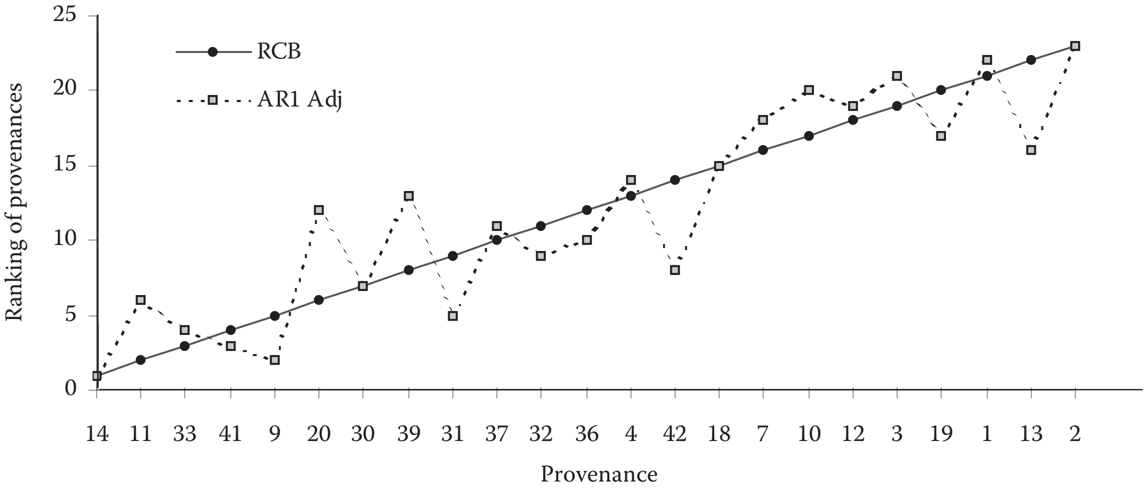

Fig. 6. Ranking of provenances relative to one another in trial 2 (Tišnov) according to RCBD and AR1 Adj

DISCUSSION

The objective of blocking is to make experimental units (e.g. provenances) as homogeneous as possible within blocks with respect to the observed variable, and to make the different blocks as heterogeneous as possible with respect to the observed variable (Neter et al. 1996). In most cases of agricultural field experiments, the intrablock homogeneity of blocks containing more than 12 plots occurs only seldom (Stroup et al. 1994). Littell et al. (1996) advocate that randomized block designs should never be used for experiments with "large" numbers of treatments. Such a marginal value is likely even smaller in forestry, because of a larger spacing between individual plants. This is in contrary to the number of provenances presented in Table 1. It therefore does not come as a surprise that blocks do not capture significant amount of variation in the observed trait (see Pr Z value in Table 2) and that alternative models have to be fitted in order to characterize the data. However, even a spatial analysis is relatively inefficient on large randomized block designs (Stroup 2002). Further complications that arise from this design are:

1. under the excessive block size there is a tendency for some treatments to be located disproportionately in relatively good or poor plots and consequently, some assumptions required by the model are not met (e.g. no interaction between treatments and blocks see Table 2 "provenance × block"),
2. small number of treatments per block require less space, leading to more homogeneous conditions and more likely to constant variance across treatment means; the opposite is true for large number of treatments in the present study,
3. multiple comparisons (conducted to compare simultaneously treatment means) are difficult to handle when large number of pair-wise tests are requested,
4. number of test plants per treatment are often planned ad hoc, leading to enormously large experiments. The site of experiment should follow prospective power calculation to control the probability of Type II error, combined with a proper choice of the experimental design.

There are modern multiple-comparison methods available within the mixed-model framework. In the current study, the number of provenances was too large for performing such a comparison in a graphically friendly way. The reader should consult Hajnala et al. (this issue) for the demonstration of these methods under more reasonable number of treatments.

Based on these results, it is obvious that the traditional randomized block design does not grant conclusive outputs because spatial patterns within trials are not taken into account (fit statistics in Table 3). Since tree breeding experiments require much more space (often more than one hectare) compared to agricultural variety crop trials, one can assume that spatial variation plays a significant role in the whole system. Dutkowski et al. (2002) advocate an initial combined model for spatial analysis of forest genetic trials, which adds an autoregressive error term to the design model and retains an independent error term. In most instances in their study, this was a considerably better model. Although not very different from the alternative models they investigated, it is simple to apply and does not inflate the additive variance. Data of Costa e Silva et al. (2001) suggest that it is essential to account for the independent error because it is always present in forestry trials, and, moreover, it is large. In variety trials with a plot as the experimental unit, independent error is assumed to represent measurement error. According to GILMOUR et al. (1997), it is often significant but usually small if it is modelled. In forestry trials, while measurement error might exist, variation from tree to tree will also be due to microsite and non-additive genetic effects (DUTKOWSKI et al. 2002). QIAO et al. (2000) compared the influence of experimental designs and spatial analyses on the estimation of genotype effects for yield (33 wheat trials) and their impact on selection decisions. The relative efficiency of the alternative designs and analyses was best measured by the average standard error of difference between line means. Both more effective designs and spatial analyses significantly improved the efficiency relative to the randomized complete block model, with the preferred model (which combined the design information and spatial trends) giving an average relative efficiency of 138% over all 33 trials. Hence, the use of these methodologies can impact on the selection decisions in plant breeding.

This agricultural example can, however, be applied in forestry trials as well. Figs. 3–6 show that before individual provenances are selected, models covering spatial variation should be tested. For instance, in trial 1, provenances #36 and #28 reached very similar predicted mean heights based on RCBD; the relative difference counts for only 1%, thus they might be regarded to have very similar features. However, when AR1 is applied, the relative difference increases to 7% and after subsequent model-fitting the difference reaches 14%. In other words, both of these provenances lie nearly in the middle of the relative ranking (Fig. 3) according to RCBD. Nevertheless, AR1 moves both of them contrariwise in the scale, and both predicted means and relative ranking change significantly. While provenance #36 drops to the worst 15 out of 50, provenance #28 appears among the best 15. This approach is therefore certainly worth considering when data from various tree breeding experiments should be processed.

Although these two provenance trials are too few to make any decisions regarding selection (the number of blocks in the first trial is small as well), these methods can, in general, substantially influence the selection process and it is the purpose of this study to point to this phenomenon rather than making strong inferences about the current trial. JOYCE et al. (2002) analyzed a farm-field test of black spruce progeny at ages 3–10 with random non-contiguous single tree plots with spatial techniques and nearest-neighbours adjustments to evaluate the effectiveness of used blocking and neighbour adjustments (4, 8 and 12 nearest neighbours) in controlling the site heterogeneity. They concluded that their results, although largely specific to one particular field test, have some general implication for genetic testing of black spruce and other forest trees: first, substantial site heterogeneity could still be found in a farm-field test, even with extensive site management and uniformity seemingly observed across a test site; second, the applied blocking could remove a proportion of a site variation, but application of more effective field design such as Alpha designs (WILLIAMS, TALBOT 1996; JOYCE et al. 2002) may help remove more site heterogeneity for higher efficiencies of genetic estimates (FU et al. 1998; JOYCE et al. 2002); third, a spatial analysis should not be overlooked for any farm-field test as it can generate useful information for assessing the effectiveness of field layouts in controlling variation (FU et al. 1999). The graphical outputs from various statistical packages such as SAS (SAS® Institute Inc. 1999) or ASREML, sample variograms, can serve as a useful diagnostic for assisting with the identification of appropriate variance models for spatial data (GILMOUR et al. 1997). JOYCE et al. (2002) describe that the neighbour adjustments displayed considerable impacts on estimates of genetic parameters associated with family rankings and genetic gains of family, individual and early selection. According to their results, the 12 nearest-neighbours used should be close to the optimal; but they suggest a further study on the choice of neighbourhood size for effective uses of neighbour adjustments. GILMOUR et al. (1997) conclude that although there is no one model that adequately fits all field experiments, the separable autoregressive model is dominant. BROWNIE and GUMPERTZ (1997) recommend fitting global trends whenever they are present. Failure to do so could lead to estimates of precision being too small. This suggestion is based on simulation studies, the aim of which was to assess validity of several correlated errors and alternative fixed effects spatial analyses. They focused on situations typical of large field trials with limited replication and realistic levels of both fixed and random components of spatial variation. As mentioned before, however, simple models should be given priority to more complicated ones because there is a risk of over-fitting effects and artificially reducing the estimates of precision.

This study has proven that spatial variation, when taken into account in forestry trials, can significantly improve the fit statistics, leading to more precise estimates of individual treatment means. Any hypothesis tests formed around these means are therefore greatly affected by the proper model selection. The two trials selected in this study were considered the "best" given the mortality and related data diagnostics. One can easily imagine that inappropriate data analysis of trials in the "worse" category could lead to huge errors in ranking of provenances and consequent false recommendations to operational forestry.

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Hodnocení provenienčních experimentů se zohledněním prostorových autokorelací na příkladu dvou ploch se smrkem černým

ABSTRAKT: Dvě provenienční plochy se smrkem černým (*Picea mariana* [Mill.] B.S.P.) byly hodnoceny s využitím tradičních statistických metod a moderních prostorových analýz. Cílem bylo vysledovat případné rozdíly mezi těmito přístupy z hlediska vhodnosti použitých modelů a také z hlediska odhadnutých průměrných výšek jednotlivých proveniencí. Prostorové modely byly následně upravovány takovým způsobem, aby se co nejlépe vypořádaly s externími zdroji proměnlivosti. Jak jsme očekávali, modely zohledňující prostorovou proměnlivost byly pro zvolené datové soubory vhodnější. Při využití těchto modelů jsme pozorovali více či méně výrazný posun nejen v odhadech průměrných výšek jednotlivých proveniencí, ale také v jejich relativním pořadí, což by mohlo ve svém důsledku významně ovlivnit i interpretaci výsledků celých provenienčních pokusů. Dále zmiňujeme problémy spojené s analýzou experimentů založených tradičním náhodným blokovým uspořádáním, kterých se využívá v lesnickém výzkumu.

Klíčová slova: *Picea mariana* (Mill.); provenienční výzkum; REML; prostorová proměnlivost

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